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(54) Recombinant interleukin-2 receptor.

EP 0 386 289 A1 (57) Recombinant IL-21R β chain or portions thereof, cDNA coding therefore, vectors containing said cDNA, hosts transfected by said vectors, and monoclonal antibodies to said recombinant IL-2R β or portions thereof.

Recombinant Protein Receptor

This invention relates to receptors for interleukin-2, more particularly to the β -chain of the receptor, and to cDNA coding for the β -chain or parts thereof, vectors containing cDNA inserts coding for the β -chain, hosts transformed by such vectors and the cultivation of such hosts to produce the said β -chain.

Ample evidence has been accumulated that cytokines, a class of soluble mediators involved in cell-to-cell "communications", are essential in the regulation of the immune system. It has been known that cytokines induce proliferation, differentiation and activation of target cells through interaction with specific cell surface receptor(s). Interleukin-2 (IL-2), previously defined as T cell growth factor (1), is one of the best characterized cytokines, known to play a pivotal role in the antigen-specific clonal proliferation of T lymphocytes (T cells) (2). IL-2 also appears to act on other cells of the immune system such as immature thymocytes (3), B lymphocytes (B cells) (4), macrophages (5), natural killer cells (NK cells) (6), and lymphokine-activated killer cells (LAK cells) (7). These multifunctional properties of IL-2 have opened now possibilities in the formulation of immunotherapies such as adoptive immunotherapy (8). More recently, IL-2 has been shown to function also on neural cells such as oligodendrocytes (9), suggesting a possible involvement of this cytokine in the central nervous system. Despite extensive studies on the IL-2 system in the context of basic and clinical immunology, information has been limited on the molecular mechanism(s) underlying the IL-2-mediated signal transduction (10).

The IL-2 receptor (IL 2R) is known to be unique in that it is present in three forms: high-, intermediate- and low-affinity forms with respect to its binding ability to IL-2, and respective dissociation constants (Kds) of 10^{-11} M, 10^{-9} M and 10^{-8} M (11, 12). Following the characterization of IL-2R α chain (Tac antigen, p55) (13), it became evident that the α chain constitutes solely the low-affinity form and it is not functional per se in IL-2 internalization and signal transduction, unless associated with another specific membrane component(s) of lymphoid cells (14, 15). Subsequently, the lymphoid membrane component was identified to be a novel receptor chain, termed β chain (or p70-75) (12, 16, 17). In fact, experimental evidence has suggested that the IL-2R β chain per se constitutes the intermediate-affinity form (12). In addition, its association with the IL-2R α chain results in the high-affinity form of the receptor (12, 16, 17). Expression studies using wild type and mutated IL-2R α chain cDNAs strongly support the notion that the IL-2R β chain but not the IL-2R α chain possesses a domain(s) responsible in driving the intracellular signal transduction pathway(s) (18). There exists therefore a need to obtain IL-2 β chain in amounts which will enable its structure and function to be elucidated, this being an essential step in gaining further insight into the molecular basis of the high-affinity IL-2R as well as on the mechanism of signal transduction operating in IL-2 responsive cells. To this end we describe below cDNA coding for the IL-2 β chain or parts thereof whereby insertion of said cDNA in a suitable vector and expression thereof in an appropriate host will enable recombinant and large scale production of protein corresponding to the IL-2 β chain or parts thereof.

Isolation and analysis of the cDNA clones

In isolating the cDNA clones, we applied an expression cloning strategy by using the monoclonal antibodies, Mik- β 1 and Mik- β 2 (19), both of which have been raised against the IL-2R β chain found on the human leukemic cell line (YT (20)). The monoclonal antibodies Mik- β 1 and Mik- β 2 are both deposited at Fermentation Research Institute, Agency of Industrial Science and Technology, Japan. The deposit numbers for Mik- β 1 and Mik- β 2 are, 10453 and 10454 (1988), respectively; they are also described in Japanese Patent Application No. 298742 (1988).

A few sets of cDNA libraries were prepared by using the poly(A)⁺-RNA from YT cells according to standard procedures. cDNA libraries were prepared with cDM8 vector according to published procedures (21), except using random primer (Amersham) or oligo (dT) primer as mentioned below. The plasmid DNA representing 5.6×10^6 independent colonies were prepared by the standard procedure and one mg of DNA were used for the first DNA transfection. Actually, the DNA was divided into 100 tubes (therefore each tube contained 10 μ g of DNA) and they were each transfected into 3.5×10^5 monkey COS cells in a tissue culture dish (60 mm polystyrene dish, Corning). The transfection was done using the standard DEAE dextran procedures. The transfected COS cells were then treated with the cocktail of Mik- β 1 and - β 2 antibodies (400-fold diluted ascites for each antibody) and subjected to the standard panning procedure. The dish used for the panning was FALCON 60 mm dish, coated with anti-mouse IgG as described previously (ref. 21). In this first round of panning, 100 IgG-coated dishes were used. After the panning, Hirt extract was prepared by the standard procedure (ref. 21) and the recovered plasmids were introduced into E.coli by the

method described in ref. 21. By this procedure 3.7×10^5 colonies were obtained. Those bacterial colonies were fused with COS cells by the standard protoplast fusion procedures (ref. 21). In these fusion experiments, 26 Corning dishes each containing 5×10^5 COS cells were used. After the fusion, the COS cells were subjected to panning as described above and Hirt extract was prepared. 32,000 bacterial colonies were obtained from the Hirt extract. The fusion, panning procedures were repeated again and 32,000 bacterial colonies were obtained from the subsequent Hirt extract. The same procedure were repeated once again, obtaining 28,000 bacterial colonies (in the meantime, there should be a dramatic enrichment of the objective clones). The same procedures were repeated once again and 6,000 colonies were obtained. From these colonies, 30 colonies were picked up randomly and the cDNA inserts were analysed. Of them, only 7 colonies contained plasmids from which cDNA inserts can be excised by restriction enzyme XhoI. The vector driven XhoI sites are located at the both side of the cDNA and all other plasmids had lost such cleavage sites due to the DNA rearrangements; in fact, all of them were much smaller in size than the original vector. Thus they were considered to be non-specific products. On the other hand, all of the 7 colonies were derived from the same mRNA, as confirmed by the conventional restriction enzyme cleavage analysis and DNA blot analysis. Of them, one plasmid, termed pIL-2R β 30 contained longer cDNA than other 6 plasmids which were turned out to be identical to each other (designated gTT-2R β 9).

In this procedure, therefore, we isolated two independent cDNA clones, pIL-2R β 9 and pIL-2R β 30; each of the expression products specifically reacted with the antibodies. The two clones contained cDNA inserts of 1.3Kb and 2.3Kb, respectively, and cross-hybridized with each other. Subsequent sequence analysis of the cDNAs revealed that they represent the same mRNA. In fact, RNA blotting analysis revealed that the mRNA is approximately 4Kb in size (see below). Subsequently, we screened other YT cDNA libraries by using the cloned cDNAs as probes, and several independent cDNA clones which together cover the entire mRNA for the IL-2R β chain were isolated. Thus pIL-2R β 6 and pIL-2R β 19 were obtained by screening the cDNA libraries with the pIL-2R β 9 cDNA insert in the probe.

The above mentioned plasmids containing cDNA coding for IL-2 β sequences have been deposited in strain E.coli MC 1061/P3 on March 2, 1989 at the Fermentation Research Institute according to the Budapest Treaty under the following accession numbers:

Plasmid	Accession No.
pIL-2R β 6	FERM BP-2312
pIL-2R β 9	FERM BP-2313
pIL-2R β 19	FERM BP-2314
pIL-2R β 30	FERM BP-2315

The complete nucleotide sequences of four of the cloned cDNAs were determined (Fig. 1).

Fig. 1 shows the structure of the human IL-2R β chain cDNA. Fig. 1a is a schematic representation of the mRNA as deduced from the cloned cDNAs. Dotted, hatched, open and closed rectangles represent respectively the signal sequence, the extracellular, the transmembrane and the cytoplasmic regions of the mRNA are shown below. Fig. 1b shows the nucleotide and amino acid sequences of the human IL-2R β chain cDNA. The sequence was deduced following the complete DNA sequence analysis of the above described cDNA clones. Nucleotides are numbered on the right margin and amino acids are numbered on the left margin. Clones pIL-2R β 19 and pIL-2R β 6 each contained G-A mutation at nucleotide residues 425 and 1531, respectively. Thus pIL-2R β 6 cDNA acquired a TAG triplet in the cytoplasmic region. It is thought to be an error in reverse transcription, since all other clones, pIL-2R β 30, pIL-2R β 19 and pIL-2R β 16 (28), have TGG triplet at that position. The first underlined 26 amino acid residues represent the signal sequence as predicted by the consensus sequence (22). The 25 transmembrane amino acid residues are marked with a thick underlining. The cysteine residues are boxed. The potential N-glycosylation sites are underlined twice. The possible poly-adenylation signals are shown by open rectangle. RNA was prepared from the NK-like human lymphoid cell line, YT, and cDNA libraries were prepared with CDM8 vector according to published procedures (21), except using either random primers (Amersham) (for pIL-2R β 6, 9 and 30), or oligo (dT) primer (for pIL-2R β 19). Screening of the cDNA libraries by a cocktail of anti-IL-2R β monoclonal antibodies, Mik- β 1 and Mik- β 2, was carried out as described previously (21). Nucleotide sequences were determined by a combination of dideoxy chain termination and chemical cleavage methods.

As shown in Fig. 1, the cDNA contains a large open reading frame that encodes a protein consisting of 551 amino acids. No significant homology with other known proteins was found in the Protein Sequence Database (National Biomedical Research Foundation, Washington, D.C.) or with sequences published more

recently. Unlike many of other cytokine receptors, it appears that IL-2R α and IL-2R β chains do not belong to the immunoglobulin superfamily. From the deduced structure of the protein, the N-terminal 26 amino acids is considered to represent the signal sequence (Fig. 1 and 2) (22). Thus the natured form of the IL-2R β chain consists of 525 amino acids with a calculated M.W. of 58.358. As shown in Fig. 1, the receptor molecule consists of an extracellular region consisting of 214 amino acids. This region contains 8 cysteine residues of which 5 residues are found in the N-terminal half and they are interspaced rather periodically by 9-12 amino acids. It is likely that disulfide linkages between the cysteine residues impart a stable configuration for ligand binding. In fact, abundance of cysteine residues seems to be one of the common features of the ligand binding domain of many receptors (23). It may be worth noting that the predicted number of amino acids (a.a.) within the extracellular region of the IL-2R β chain (214 a.a.) is almost comparable in number to that of the IL-2R α chain (219 a.a.). Such size similarity may be significant in considering the conformation of the heterodimeric receptor complex that is quite unique for this receptor; as both α and β chains individually interact with distinct sites of the same IL-2 molecule (24).

A hydrophobic stretch of 25 amino acids spanning from the 215 to 239 amino acid residues appears to constitute the membrane spanning region of the receptor (Fig. 1 and 2).

Fig. 2 is a hydropathy plot analysis of deduced human IL-2R α and IL-2R β chain precursor structures. The analysis was carried out according to Kyte and Doolittle (38). SG and TM each represents signal sequence and transmembrane sequence, respectively.

The cytoplasmic region of the β chain consists of 286 a.a. and it is far larger than that of the α chain, which is only 13 a.a. long. The consensus sequences of tyrosine kinase (Gly-x-Gly-x-x-Gly) (25) are absent in the β chain. However, the presence of a triplet, Ala-Pro-Glu (293-295) may be noted; this has been asserted to be the consensus motif for a catalytic domain of some protein kinases (25). The possibility of the cytoplasmic region of the β chain having a protein kinase activity has yet to be tested. The primary structure of this region revealed yet another interesting feature; a rather strong bias for certain characteristic amino acids. This region is rich in proline (42/286) and serine (30/286) residues. Interestingly, the "proline rich" structure has also been demonstrated in the cytoplasmic region of CD2, a T cell membrane antigen involved in the activation pathway of T cells (26). The proline-rich structure may impart a non-globular conformation to this region that may be important in coupling of the receptor molecule with other signal transducer(s). The predominant serine residues may be the major target for phosphorylation, which could also modulate the receptor function (27). In addition, the cytoplasmic region is notably biased for negatively charged amino acids. In fact, this region contains 40 such amino acids (i.e. glutamic and aspartic acids), whereas only 18 amino acids account for the positively charged residues (i.e. lysine and arginine). Such a bias is particularly notable in the middle portion (a.a. 345-390) of the cytoplasmic region. Thus, the cytoplasmic region of the β chain may be quite acidic. Taken together some if not all of these unique characteristics may be responsible in driving further the downstream signal transduction pathway(s). The receptor protein contains 5 potential sites for N-linked glycosylation (Fig. 1), in which 4 are located in the extracellular region. Such a posttranslational modification may account for the difference between the M.W. of the estimated mature (70-75kD) and the calculated (58kD) protein molecules. Hydropathy plot analysis of the α and β chains revealed the presence of hydrophilic regions just adjacent to the cell membrane in the both chains (Fig. 2) These regions may play a role in the non-covalent intramolecular association between the two chains.

According to a broad aspect of the present invention therefore we provide a recombinant cDNA coding for the IL-2R β chain.

Preferably the cDNA is defined by a structural gene having formula:

GCAGCCAGAGCTCAGCAGGGCCCTGGAGAGATGG

CCACGGTCCCAGCACCGGGGAGGACTGGAGAGCGCGCGCTGCCACCGCCCC

5

ATGTCTCAGCCAGGGCTTCCTTCCTCGGCTCCACCCTGTGGATGTA ATG

GCG GCC CQT GCT CTG TCC TGG CGT CTG CCC CTC CTC ATC

CTC CTC CTG CCC CTG GCT ACC TCT TGG GCA TCT GCA GCG

GTG AAT GGC ACT TCC CAG TTC AGA TGC TTC TAC AAC TCG

10

AGA GCC AAC ATC TCC TGT CTC TGG AGC CAA GAT GGG GCT

CTG CAG GAC ACT TCC TGC CAA GTC CAT GCC TGG CCG GAC

AGA CGG CGG TGG AAC CAA ACC TGT GAG CTG CTC CCC GTG

15

AGT CAA GCA TCC TGG GCC TGC AAC CTG ATC CTC GGA GCC

CCA GAT TCT CAG AAA CTG ACC ACA GTT GAC ATC GTC ACC

CTG AGG GTG CTG TGC CGT GAG GGG GTG CGA TGG AGG GTG

ATG GCC ATC CAG GAC TTC AAG CCC TTT GAG AAC CTT CGC

20

CTG ATG GCC CCC ATC TCC CTC CAA GTT GTC CAC GTG GAG

ACC CAC AGA TGG AAC ATA AGC TGG GAA ATC TCC CAA GCC

TCC CAC TAC TTT GAA AGA CAC CTG GAG TTC GAG GCC CGG

25

ACG CTG TCC CCA GGC CAC ACC TGG GAG GAG GCC CCC CTG

CTG ACT CTC AAG CAG AAG CAG GAA TGG ATC TGC CTG GAG

ACG CTC ACC CCA GAC ACC CAG TAT GAG TTT CAG GTG CGG

GTC AAG CCT CTG CAA GGC GAG TTC ACG ACC TGG AGC CCC

30

TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC

CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC

GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG

35

TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG

AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG

TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC

CAG AAG TGG CTC TCT TCG CCC TTC CCC TGA TCG TCC TTC

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AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA

GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG

CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC

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CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC

TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC

CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC

CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC

50

CCC CAA CCC CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC

TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC

TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT

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GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC
CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC
CAG CCC CTG GGG CCT CCC ACC CCA GGA GTC CCA GAC CTG
GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG
GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA
GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG
TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT
GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA
ATC CAC TTG GTG TAG ACAGATGGCCAGGGTGGGAGGCAGGCAGCT
GCCTGCTCTGCGCCGAGCCTCAGAAGGACCCTGTTGAGGGTCCTCAGTCCA
CTGCTGAGGACACTCAGTGTCCAGTTGCAGCTGGACTTCTCCACCCGGATG
GCCCCACCCAGTCCTGCACACTTGGTCCATCCATTTCCAAACCTCCACTG
CTGCTCCCGGGTCTGCTGCCCCGAGCCAGGAAGTGTGTGTGTTGCAGGGGG
GCAGTAACTCCCCAACTCCCTCGTTAATCACAGGATCCCACGAATTTAGGC
TCAGAAGCATCGCTCCTCTCCAGCCCTGCAGCTATTCACCAATATCAGTCC
TCGCGGCTCTCCAGGGCTCCCTGCCCTGACCTCTTCCCTGGGTTTTCTGCC
CCAGCCTCCTCCTTCCCTCCCCCTCCCCGTCCACAGGGCAGCCTGAGCGTGC
TTTCCAAAACCCAAATATGGCCACGCTCCCCCTCGGTTCAAACCTTGCAC
AGGTCCCACTGCCCTCAGCCCCACTTCTCAGCCTGGTACTTGTACCTCCGG
TGTCGTGTGGGGACATCCCCCTTGCAATCCTCCCTACCGTCCCTCCCGAGC
CACTCAGAGCTCCCTCACACCCCCTCTGTTGCACATGCTATTCCTGGGGC
TGCTGTGCGCTCCCCCTCATCTAGGTGACAACTTCCCTGACTCTTCAAGT
GCCGGTTTTGCTTCTCCTGGAGGGAAGCACTGCCTCCCTTAATCTGCCAGA
AACTTCTAGCGTCAGTGCTGGAGGGAGAAGCTGTCAGGGACCCAGGGCGCC
TGGAGAAAGAGGCCCTGTTACTATTCTTTGGGATCTCTGAGGCCTCAGAG
TGCTTGGCTGCTGTATCTTTAATGCTGGGGCCCAAGTAAGGGCACAGATCC
CCCCGACAAAGTGGATGCCTGCTGCATCTTCCACAGTGGCTTCACAGACC
CACAAGAGAAGCTGATGGGGAGTAAACCCTGGAGTCCGAGGCCCAGGCAGC
AGCCCCGCCTAGTGGTGGGCCCTGATGCTGCCAGGCCTGGGACCTCCCACT
GCCCCCTCCACTGGAGGGGTCTCCTCTGCAGCTCAGGGACTGGCACACTGG
CCTCCAGAAGGGCAGCTCCACAGGGCAGGGCCTCATTATTTTTCACTGCCC
CAGACACAGTGCCCAACACCCCGTCGTATACCCTGGATGAACGAATTAATT
ACCTGGCACCACTCGTCTGGGCTCCCTGCGCCTGACATTCACACAGAGAG
GCAGAGTCCCGTGCCCATTAGGTCTGGCATGCCCCCTCCTGCAAGGGGCTC
AACCCCTTACCCCGACCCCTCCACGTATCTTTCTAGGCAGATCACGTTGC
AATGGCTCAAACAACATTCCACCCCAGCAGGACAGTGACCCCACTCCAGC
TAACTCTGACCTGGGAGCCCTCAGGCACCTGCACTTACAGGCCTTGCTCAC

5 AGCTGATTGGGCACCTGACCACACGCCCCACAGGCTCTGACCAGCAGCCT
 ATGAGGGGGTTTGGCACCAAGCTCTGTCCAATCAGGTAGGCTGGGCCTGAA
 CTAGCCAATCAGATCAACTCTGTCTTGGGCGTTTGAACCTCAGGGAGGGAGG
 CCCTTGGGAGCAGGTGCTTGTGGACAAGGCTCCACAAGCGTTGAGCCTTGG
 10 AAAGGTAGACAAGCGTTGAGCCACTAAGCAGAGGACCTTGGGTTCCCAATA
 CAAAAATACCTACTGCTGAGAGGGCTGCTGACCATTGTCAGGATTCCTG
 TTGCCTTTATATCCAAAATAAACTCCCCTTTCTTGAGGTTGTCTGAGTCTT
 15 GGGTCTATGCCTTGAAAAAGCTGAATTATTGGACAGTCTCACCTCCTGCC
 ATAGGGTCCTGAATGTTTCAGACCACAAGGGGCTCCACACCTTTGCTGTGT
 GTTCTGGGGCAACCTACTAATCCTCTCTGCAAGTCGGTCTCCTTATCCCCC
 CAAATGGAAATTGTATTTGCCTTCTCCACTTTGGGAGGCTCCCACTTCTTG
 20 GGAGGGTTACATTTTTTAAGTCTTAATCATTGTTGACATATGTATCTATAC
 ATCCGTATCTTTTAATGATCCGTGTGTACCATCTTTGTGATTATTTCTTA
 ATATTTTTTCTTTAAGTCAGTTCATTTTCGTTGAAATACATTTATAAAGAA
 25 AAATCTTTGTTACTCTGTAAATGAAAAACCCATTTTCGCTATAAATAAAA
 GGTAACGTACAAAATAAGTACAAT

30 The present invention also includes cDNA coding for portions of the complete sequence of the IL-2R β chain for instance the extracellular portion beginning at, or about amino acid (a.a) 1 e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and ending at or about a.a. 214 e.g. 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 214, 215, 216, 217, 218, 219, 220, or sub-portions of this extracellular part, or portions corresponding to the
 35 intracellular part of the receptor chain e.g. the portion beginning at or about a.a. 239 e.g. a.a. 230, 231, 232, 234, 235, 236, 237, 238, 239, 240, 241, 242, up to or about the end a.a. 525, e.g. 516, 517, 518, 519, 520, 521, 522, 523, 524 and 535.

40 Using standard techniques of recombinant DNA technology vectors for transforming suitable host cells can be constructed which contain cDNA sequences corresponding to the structural gene for IL-2R β as set forth above or any desired portion thereof, or a degenerate variant thereof.

Suitable vectors are plasmid vectors for example and will include control and regulatory sequences operably linked to the cDNA sequence coding for the IL-2R β chain or portion thereof.

Suitable techniques are well known and widely practised and by way of Example are described, in connection with other proteins in European Patent Applications, Publication Nos. 0254249 and 0170204.

45 Obtaining the desired portion in pure form from the culture can be carried out by standard techniques and such protein provides a suitable antigen for preparing monoclonal antibodies. Thus hybridomas capable of secreting a monoclonal antibody having a specific affinity to the IL-2R β chain or a desired portion thereof may be prepared by immunizing a non-human animal with recombinant IL-2R β or a portion thereof, removing spleen cells with non-immunoglobulin secreting myelomas cells, and selecting from the resulting
 50 hybridomas a cell line which produces a monoclonal antibody having the desired binding specificity and, if desired, subsequently sub-cloning said hybridoma.

The techniques for preparing hybridomas and obtaining monoclonal antibodies in pure form therefrom are well known and by way of example are described in European Patent Application, Publication No. 0168745.

55 Antibodies in accordance with the invention are useful e.g. for diagnostic purposes and also for therapy by immune suppression or activation. As mentioned above, such antibodies could be raised using purified recombinant protein in accordance with the invention or by transfecting the cDNA of the invention, obtaining cells expressing large amounts of the receptor and using such cells to obtain the antibodies.

The present invention envisages soluble forms of IL-2R β chain and of soluble IL-2 receptor. That is the IL-2R β chain may be produced in soluble form or the α -chain and β -chain produced simultaneously.

The availability of monoclonal antibodies to specific sub-portions of the IL-2 β chain enables epitopes of the receptor chain to be identified and thus opens the way for control of the activity of the receptor to be exercised using suitable monoclonal antibodies or other peptides or peptide mimetic or protein analogues substances.

Expression of IL-2R β chain mRNA

Expression of the IL-2R β mRNA was examined by using the cDNA insert from pIL-2R β 30 as the probe

Fig. 3a illustrates the expression of human IL-2R β chain mRNA in different cell types. Poly(A)⁺ RNA (2 μ g per lane) from the following cell sources was prepared and subjected to RNA blotting analysis using the XhoI-digested human IL-2R β chain cDNA fragment derived from pIL-2R β 30 as a probe following standard procedures (14, 18, 27). Lane 1, YT; lane 2, Hut102(HTLV-1 transformed human T cell line); lane 3, MT-2(HTLV-1 transformed human T cell line); lane 4, ARH-77 (multiple myeloma line); lane 5, SKW6.4 (EBV-transformed human B lymphoblastoid line); lane 6, U937 (histiocytic leukemia line); lane 7, MT-1 (HTLV-1 transformed human T cell line); lane 8, Jurkat (human T leukemic line); lane 9, HeLa (human cervical carcinoma cell line).

As shown in Figure 3a, the RNA blot analysis revealed the presence of a 4kb mRNA, the expression of which is restricted to lymphoid cells previously identified to bear IL-2R β chain (i.e. YT, MT-2, Hut102, SKW6.4) (12, 16, 17). On the other hand, the mRNA expression was not detected in cells such as Jurkat, MT-1, U937, ARH-77 and HeLa cells. Essentially, the mRNA expression levels are in correlation with the IL-2R β chain expression levels.

Fig. 3 b illustrates the expression of IL-2R β and IL-2R α mRNAs in human PBLs. Total RNA (15 μ g per lane) was loaded in each lane. Lanes 1 and 4 represents unstimulated human peripheral blood lymphocytes (PBLs); lanes 2 and 5, PBLs stimulated with 5 μ g/ml phytohemagglutinin (PHA) for 24 hrs; lanes 3 and 6, PBLs stimulated with 5 μ g/ml PHA for 72 hrs. The RNA-blotted filter was hybridized with the IL-2R β probe (lanes 1-3). After dehybridization of the IL-2R β probe, the same filter was hybridized with the IL-2R α probe (XbaI-BclI fragment derived from pSVIL2R-3 (14) (lanes 4-6).

Interestingly, the IL-2R β mRNA was detectable in the unstimulated PBLs and its expression levels increased transiently only 2.5-fold after mitogen stimulation. Based on previous data derived from flow cytometric analysis (19), it is likely that the mRNA induction patterns differ between the different lymphocyte populations. This expression pattern is quite different from that of the IL-2R α chain whose expression strictly requires mitogenic stimulation of the cells (Fig. 3b), suggesting the presence of distinct mechanisms of gene expression between the two genes.

Southern blot analysis of the genomic DNA from PBL and various cell lines including HTLV-1-transformed human T cell lines indicates that the gene is present in a single copy and is not rearranged in those cells.

IL-2 binding properties of the cDNA-encoded IL-2R β chain

We next carried out a series of cDNA expression studies in order to examine if the cDNA product binds IL-2 and indeed manifests the properties of the IL-2R β chain that have been demonstrated and/or suggested in previous studies. Two cDNA expression plasmids were constructed in which expression of the cDNA spanning the entire coding region was directed by either the mouse *lck* gene (29) promoter (pLCKR β) or Moloney leukemia virus LTR (30) (pMLVR β).

Expression vectors were constructed by the following procedures. pIL-2R β 30 was digested with HindIII (the cleavage site is located within the polylinker regions of CDM8) and, after fill-in of both ends, a BamHI linker was attached and religated. The resulting plasmid was then digested with BamHI and the 1.8kb DNA fragment which contains the entire coding sequence for the β chain was introduced into BamHI-cleaved p1013 vector containing the mouse *lck* promoter to construct pLCKR β . The BamHI-digested cDNA fragment was also introduced into a retrovirus vector, pZipSV(X) (30), to construct pMLVR β . The human IL-2R α expressing vector, pSVIL2Rneo, was obtained from pSVIL2R-3 (14) by replacing the Eco-gypt gene with the neo-resistance gene.

The plasmid pLCKR β was introduced into the mouse T lymphoma EL-4 and the human T cell leukemia Jurkat lines, both of which are known to be devoid of surface molecules that bind human IL-2.

Transfection of the expression plasmids into Jurkat and EL-4 cells was carried out by electroporation as described previously (39). Transfected cells were selected in the RPMI1640 medium containing 10% fetal calf serum (FCS) and G418 (1 mg/ml for EL-4 and 1.5 mg/ml for Jurkat). To obtain cells expressing cDNAs for human IL-2R α and IL-2R β chains simultaneously, a Jurkat-derived clone J α -5, transfected with pSVIL2Rneo, was co-transfected with pLCKRB and a plasmid containing the hygromycin-resistance gene, pHgy. The transfected cells were selected with 200 μ g/ml hygromycin. Transfection of pMLVR β into 2 cells was carried out by calcium-phosphate method as described previously (14) and the cells were selected by 700 μ g/ml of G418. For flow cytometric analysis, 5 \times 10⁵ cells were treated with antibody (1:500 dilution of ascites) at 4 °C for 30 min. After washing, cells were stained with fluorescein-conjugated goat anti-mouse IgG.

The stained cells were analysed on a FACS440 flow cytometer (Beckton Dickinson). The ¹²⁵I-IL-2 binding assay and Scatchard plot analysis were carried out as described previously (12).

Fig. 4a illustrates the expression of human IL-2R α and/or IL-2R β chain cDNAs by means of cell surface staining patterns of human IL-2R α and/or IL-2R β cDNA transfectants. Parental cells and various transfectant cells were separately stained with either a monoclonal anti-human IL-2R α antibody, anti-Tac (----), or monoclonal anti-human IL-2R β antibody, Mik-B1 (————), Dotted line (.....) is a fluorescence profile of the cells stained with fluorescein-conjugated goat-anti-mouse IgG alone. Cells used were (1) ELB-13 (and EL-4-derived clone transfected with pLCKR β), (2) J β -8 (a Jurkat-derived clone transfected with pLCKR β), (3) J α -5 (a Jurkat-derived clone transfected with pSVIL2Rneo), (4) J α -2 (a J α -5-derived clone transfected with pLCKR β), (5) J $\alpha\beta$ -10 (a J α -5-derived clone transfected with pLCKR β), and (6) FB-3 (a NIH3T3-derived line transfected with pMLVR β).

Stable transformant clones expressing the cDNA product were obtained for both the EL-4 (EL β -13) and Jurkat (J β -8 and J β -9) cells as judged by FACS analysis (Fig. 4a). In addition, we also introduced the same gene into the Jurkat transformant clone, J α -5, which expresses the transfected, human IL-2R α chain cDNA. Two of the resulting transformants, J $\alpha\beta$ -2 and J $\alpha\beta$ -10, were found to express both α and β chains (Fig. 4a-(4), (5)). As expected, RNA blotting analyses of the mRNA expressed in those transformants revealed that the α and β chain-specific mRNAs are derived from the transfected cDNAs but not from the endogeneous genes (26). Furthermore, in order to examine the property of the cDNA product in non-lymphoid cells, the plasmid pMLVR β was introduced into an NIH3T3 cell-derived cell line 2 (30), and the resulting transformant expressing the cDNA, F β -3, was obtained (Fig. 4a-(5)).

The IL-2 binding studies were carried out with ¹²⁵I-labeled, recombinant human IL-2.

Fig. 4b illustrates the expression of the α and β chains by means of the Scatchard plot analysis of ¹²⁵I-IL-2 binding to the transfectants expressing the cloned cDNAs. Scatchard plot of the IL-2 binding data in the absence (○—○) or presence (●—●) of 1:100-diluted ascites of Mik- β 1. Binding of ¹²⁵I-IL-2 to EL β -13 or J β -8 was completely abolished by Mik- β 1. No specific IL-2 binding was observed when parental Jurkat or EL-4 cells were examined. The number of IL-2 binding sites per cell and the receptor affinity were determined by computer-assisted analysis of the IL-2 binding data. (1) EL β -13, (2) J β -8, (3) J α -5, (4) J $\alpha\beta$ -2, (5) J $\alpha\beta$ -10.

As can be seen the EL-4-derived clone (EL β -13) and the Jurkat-derived clone (J β -8), both expressing the β chain cDNA displayed intermediate-affinity to IL-2 with estimated K_d values of 4.0nM and 2.7nM, respectively. The IL-2 binding to those cells was completely abolished by the Mik- β 1 antibody (Fig. 4b-(1), (2)). The Jurkat-derived J $\alpha\beta$ -2 and J $\alpha\beta$ -10 clones expressing both the human IL-2R α and IL-2R β cDNA displayed both high and low affinity receptors with estimated K_p values of 22pM and 15nM for J $\alpha\beta$ -2 and 19pM and 33nM for J $\alpha\beta$ -10, respectively. In contrast, the parental, Jurkat-derived J α -5 cells expressing the α chain cDNA alone manifested exclusively low-affinity (K_d: 19.5nM) to IL-2 (Fig. 4b-(3)). the number of the high-affinity IL-2R expressed J $\alpha\beta$ -2 cells and J $\alpha\beta$ -10 was comparable to that of expressed IL-2R β molecules. In addition, treatment of these cells with Mik- β 1 antibody completely abolished high-affinity IL-2 binding sites from the cell surface, while retaining the expression of low-affinity IL-2R (Fig. 4b-(4), (5)). These observations demonstrate unequivocally that the cDNA-encoded IL-2R β molecule is directly involved in the formation of high-affinity receptor complex in association with the IL-2R α chain. In contrast to the aforescribed T cell transformants, the F β -3 cells did not display any IL-2 binding on the cell surface under same binding conditions. Interestingly the same observation was made with monkey COS cells that express the β chain, but failed to bind IL-2 (28). Thus, the results suggest the involvement of either a cell-type specific processing mechanism(s) or an additional cellular component(s), or both for the functional IL-2R β chain expression.

In order to characterize further the molecular structure of reconstituted IL-2R, we performed chemical crosslinking experiments with ¹²⁵I-IL-2 and non-cleavable chemical crosslinker, disuccinimidyl suberate (DSS).

Fig. 5 illustrates the results of the affinity cross-linking studies of the IL-2R-positive transformants. Cells were incubated with 5nM (lanes 1-13) or 100pM (lanes 14-16) of 125 I-IL-2 in the absence (lanes 1-4, 14-16) or presence of a 250-fold molar excess of unlabeled IL-2 (lanes 5-7), 500-fold molar excess of affinity column-purified Mik- β 1 (lanes 8-10) or 500-fold molar excess of affinity column-purified anti-Tac (lanes 11-13). Then cells were chemically crosslinked with disuccinimidyl suberate (DSS) as described previously (16). The cells were then solubilized and the supernatants were subjected to 7.5% SDS-PAGE. Cells used were: Jurkat (lane 1); J α -5 (lanes 2, 5, 8, 11, 14); J β -8 (lanes 3, 6, 9, 12, 15); J $\alpha\beta$ -10 (lanes 4, 7, 10, 13, 16). YT cells crosslinked with 125 I-IL-2 were used as a marker (M).

As can be seen cells expressing only IL-2R β chain were crosslinked with 125 I-labeled IL-2 and analysed by SDS-PAGE, a doublet band consisting of 90kD major and 85kD minor was detected and its migration profile was indistinguishable from that of YT cells (see arrows in Fig. 5 and ref. 16, 17). The appearance of the doublet is inhibited by an excess of unlabeled IL-2 or by Mik- β 1. The doublet formation may be due to degradation of receptor-IL-2 complex. It is also possible that both protein products are derived by a differential post-translational modification(s). Alternatively, one of the doublet may represent a third component of the receptor complex. A broad band migrating around the position of 150kD was also detected in the transfectant (J $\alpha\beta$ -10) as well as YT cells. The appearance of this band is also inhibited by either unlabeled IL-2 or Mik- β 1. It may represent the ternary complex of IL-2, IL-2R α and IL-2R β molecules. In a series of chemical cross-linking experiments shown in Fig. 4, it was demonstrated that the physico-chemical properties of the receptor complex expressed on the surface of J $\alpha\beta$ -2 are indistinguishable from the properties of high-affinity receptor expressed on cultured T cells or PBLs (12, 16, 17).

Preliminary results of experiments to determine whether the expression of the α and β chains in non-lymphoid cells results in the formation of high-affinity receptor indicate that, when the α and β chain cDNAs are co-expressed transiently in COS cells, both chains can crosslink with 125 I-IL-2 at the concentration (400 pM) in which the similarly expressed α chain alone can not (28). The results may suggest the formation of the $\alpha\beta$ heterodimeric receptor in this non-lymphoid cell line.

IL-2 internalization by reconstituted receptors

It has been reported that intermediate- and high-affinity IL-2 receptors can both internalize IL-2 (33-35). Ligand internalization is usually accompanied with the IL-2 signal transduction, suggesting this process to be essential.

Fig. 6 illustrates IL-2 internalization via the reconstituted receptors. IL-2 internalization was examined according to a method described previously (33). Briefly, cells (5×10^7) were treated with 125 I-IL-2 at a final concentration of 200pM (J $\alpha\beta$ -10) or 5nM (J α -5, J β -8 and EL β -13) at 0°C for 30 min. After washing, cells were suspended with prewarmed culture medium (37°C) and the kinetics of IL-2 internalization was examined as described previously (33). (a) EL β -13, (b) J β -8, (c) J $\alpha\beta$ -10, (d) J α -5. (●-●-●-), internalized IL-2; (...O...O...), cell-surface bound IL-2; (■-■-■-), free IL-2.

As shown in Fig. 6, we examined whether the reconstituted receptors can internalize IL-2. In fact, the cells expressing IL-2R β chain alone, or both α and β chains are capable of internalizing IL-2 following a kinetic pattern similar to that reported for the native receptor. In contrast, the Jurkat cells expressing only IL-2R α failed to internalize IL-2, similar to previously reported observations (33, 34). Preliminary results indicate that the growth of the cells expressing the intermediate- or high-affinity receptors is selectively inhibited by IL-2 (14, 36). We also have preliminary results that the β chain expressed in another host cell line functions in stimulating the cell growth in response to IL-2 (28).

The availability of the gene encoding the IL-2R β chain makes it possible to explore novel approaches for the functional studies of the IL-2 system. The receptor structure operating in the IL-2 system is unique in that two structurally distinct membrane molecules, the IL-2R α and IL-2R β chains, both bind IL-2 independently. The series of cDNA expression examples described herein substantiate further the previous notion that the α and β chains constitute the high-affinity IL-2R complex via a non-covalent association of the molecules (18, 37). Thus the peculiarity of this system is the involvement of three intermolecular interactions between one ligand and two distinct receptors. By virtue of the present invention it will now be possible to elucidate functional domains of this unique cytokine receptor system. Mutational analyses of the cloned β chain cDNA may provide clues as to the identification of respective domains involved in ligand binding and association with the α chain. To date, little is known about the cascade of biochemical events triggered by cytokines interacting with their homologous receptors. By the present invention we have demonstrated the presence in the IL-2R β chain of a large cytoplasmic region which most likely is involved in driving the IL-2 signal pathway(s). The particular acidic nuclei found in the cytoplasmic region may suggest

coupling to other cytoplasmic signal transducers. Alternatively, in view of a previous report on the presence of IL-2 within the nucleus (33), an intriguing possibility is that the acidic as well as the proline-rich regions of the IL-2R β cytoplasmic component may play a role in activation of the genetic programming. The availability of the expression system in which the cDNA-encoded β chain can deliver growth signals will allow further clarification of the functional domains of the receptor. It is now possible to study the essential role of IL-2 in the development and regulation of the immune system.

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Claims

1. A recombinant DNA molecule coding for the β -chain of the IL-2 receptor or a portion thereof.
2. A recombinant DNA molecule characterized by a structural gene having the formula:

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ATG

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GCG GCC CCT GCT CTG TCC TGG CGT CTG CCC CTC CTC ATC
 CTC CTC CTG CCC CTG GCT ACC TCT TGG GCA TCT GCA GCG
 GTG AAT GGC ACT TCC CAG TTC AGA TGC TTC TAC AAC TCG
 AGA GCC AAC ATC TCC TGT CTC TGG AGC CAA GAT GGG GCT
 CTG CAG GAC ACT TCC TGC CAA GTC CAT GCC TGG CCG GAC
 AGA CGG CGG TGG AAC CAA ACC TGT GAG CTG CTC CCC GTG
 AGT CAA GCA TCC TGG GCC TGC AAC CTG ATC CTC GGA GCC
 CCA GAT TCT CAG AAA CTG ACC ACA GTT GAC ATC GTC ACC
 CTG AGG GTG CTG TGC CGT GAG GGG GTG CGA TGG AGG GTG
 ATG GCC ATC CAG GAC TTC AAG CCC TTT GAG AAC CTT CGC
 CTG ATG GCC CCC ATC TCC CTC CAA GTT GTC CAC GTG GAG
 ACC CAC AGA TGG AAC ATA AGC TGG GAA ATC TCC CAA GCC
 TCC CAC TAC TTT GAA AGA CAC CTG GAG TTC GAG GCC CGG
 ACG CTG TCC CCA GGC CAC ACC TGG GAG GAG GCC CCC CTG
 CTG ACT CTC AAG CAG AAG CAG GAA TGG ATC TGC CTG GAG
 ACG CTC ACC CCA GAC ACC CAG TAT GAG TTT CAG GTG CGG
 GTC AAG CCT CTG CAA GGC GAG TTC ACG ACC TGG AGC CCC
 TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC
 CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC
 GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG
 TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG
 AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG
 TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC
 CAG AAG TGG CTC TCT TCG CCC TTC CCC TGA TCG TCC TTC
 AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA
 GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG
 CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC
 CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC

5 TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC
 CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC
 CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC
 CCC CAA CCG CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC
 TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC
 10 TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT
 GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC
 CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC
 CAG CCC CTG GGG CCT CCC ACC CCA GGA GTC CCA GAC CTG
 15 GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG
 GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA
 GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG
 20 TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT
 GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA
 ATC CAC TTG GTG TAG

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or a portion thereof or a degenerate variant thereof.

3. A recombinant DNA molecule according to claim 2 characterized by a DNA sequence having the formula:

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GCAGCCAGAGCTCAGCAGGGCCCTGGAGAGATGG
 CCACGGTCCCAGCACCGGGGAGGACTGGAGAGCGCGCGCTGCCACCGCCCC
 ATGTCTCAGCCAGGGCTTCCTTCCTCGGCTCCACCCTGTGGATGTA ATG
 35 GCG GCC CCT GCT CTG TCC TGG CGT CTG CCC CTC CTC ATC
 CTC CTC CTG CCC CTG GCT ACC TCT TGG GCA TCT GCA GCG
 GTG AAT GGC ACT TCC CAG TTC AGA TGC TTC TAC AAC TCG
 40 AGA GCC AAC ATC TCC TGT CTC TGG AGC CAA GAT GGG GCT
 CTG CAG GAC ACT TCC TGC CAA GTC CAT GCC TGG CCG GAC
 AGA CGG CGG TGG AAC CAA ACC TGT GAG CTG CTC CCC GTG
 AGT CAA GCA TCC TGG GCC TGC AAC CTG ATC CTC GGA GCC
 45 CCA GAT TCT CAG AAA CTG ACC ACA GTT GAC ATC GTC ACC
 CTG AGG GTG CTG TGC CGT GAG GGG GTG CGA TGG AGG GTG
 ATG GCC ATC CAG GAC TTC AAG CCC TTT GAG AAC CTT CGC
 50 CTG ATG GCC CCC ATC TCC CTC CAA GTT GTC CAC GTG GAG
 ACC CAC AGA TGG AAC ATA AGC TGG GAA ATC TCC CAA GCC
 TCC CAC TAC TTT GAA AGA CAC CTG GAG TTC GAG GCC CGG

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ACG CTG TCC CCA GGC CAC ACC TGG GAG GAG GCC CCC CTG
 CTG ACT CTC AAG CAG AAG CAG GAA TGG ATC TGC CTG GAG
 ACG CTC ACC CCA GAC ACC CAG TAT GAG TTT CAG GTG CGG
 GTC AAG GCT CTG CAA GGC GAG TTC ACG ACC TGG AGC CCC
 TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC
 CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC
 GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG
 TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG
 AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG
 TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC
 CAG AAG TGG CTC TCT TCG CCC TTC CCC TGA TCG TCC TTC
 AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA
 GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG
 CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC
 CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC
 TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC
 CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC
 CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC
 CCC CAA CCC CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC
 TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC
 TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT
 GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC
 CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC
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 GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG
 GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA
 GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG
 TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT
 GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA
 ATC CAC TTG GTG TAG ACAGATGGCCAGGGTGGGAGGCAGGCAGCT
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CCAGCCTCCTCCTTCCCTCCCCCTCCCCGTCCACAGGGCAGCCTGAGCGTGC
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10 GCCGGTTTTGCTTCTCCTGGAGGGAAGCACTGCCTCCCTTAATCTGCCAGA
AACTTCTAGCGTCAGTGCTGGAGGGAGAAGCTGTCAGGGACCCAGGGCGCC
TGGAGAAAGAGGCCCTGTTACTATTCCCTTGGGATCTCTGAGGCCTCAGAG
15 TGCTTGGCTGCTGTATCTTTAATGCTGGGGCCCAAGTAAGGGCACAGATCC
CCCCGACAAAGTGGATGCCTGCTGCATCTTCCCACAGTGGCTTCACAGACC
CACAAGAGAAGCTGATGGGGAGTAAACCCTGGAGTCCGAGGCCAGGCAGC
AGCCCCGCCTAGTGGTGGGCCCTGATGCTGCCAGGCCTGGGACCTCCCACT
20 GCCCCCTCCACTGGAGGGGTCTCCTCTGCAGCTCAGGGACTGGCACACTGG
CCTCCAGAAGGGCAGCTCCACAGGGCAGGGCCTCATTATTTTCACTGCCC
CAGACACAGTGCCCAACACCCCGTCGTATACCCTGGATGAACGAATTAATT
25 ACCTGGCACACCTCGTCTGGGCTCCCTGCGCCTGACATTCACACAGAGAG
GCAGAGTCCCGTGCCCATTAGGTCTGGCATGCCCCCTCCTGCAAGGGGCTC
AACCCCCCTACCCCGACCCCTCCACGTATCTTTCCTAGGCAGATCACGTTGC
AATGGCTCAAACAACATTCCACCCCAGCAGGACAGTGACCCAGTCCCAGC
30 TAACTCTGACCTGGGAGCCCTCAGGCACCTGCACTTACAGGCCTTGCTCAC
AGCTGATTGGGCACCTGACCACACGCCCCCACAGGCTCTGACCAGCAGCCT
ATGAGGGGGTTTGGCACCAAGCTCTGTCCAATCAGGTAGGCTGGGCCTGAA
35 CTAGCCAATCAGATCAACTCTGTCTTGGGCGTTTGAATCAGGGAGGGAGG
CCCTTGGGAGCAGGTGCTTGTGGACAAGGCTCCACAAGCGTTGAGCCTTGG
AAAGGTAGACAAGCGTTGAGCCACTAAGCAGAGGACCTTGGGTTCCCAATA
CAAAAATACCTACTGCTGAGAGGGCTGCTGACCATTGTTGTCAGGATTCCTG
40 TTGCCTTTATATCCAAAATAAACTCCCCCTTCTTGAGGTTGTCTGAGTCTT
GGGTCTATGCCTTGAAAAAAGCTGAATTATTGGACAGTCTCACCTCCTGCC
ATAGGGTCCTGAATGTTTCAGACCACAAGGGGCTCCACACCTTTGCTGTGT
45 GTTCTGGGGCAACCTACTAATCCTCTCTGCAAGTCGGTCTCCTTATCCCCC
CAAATGGAAATTGTATTTGCCTTCTCCACTTGGGAGGCTCCCACTTCTTG
GGAGGGTTACATTTTTTAAGTCTTAATCATTTGTGACATATGTATCTATAC
50 ATCCGTATCTTTAATGATCCGTGTGTACCATCTTTGTGATTATTTCCCTTA
ATATTTTTTCTTTAAGTCAGTTCATTTTCGTTGAAATACATTTATAAAGAA
AAATCTTTGTTACTCTGTAAATGAAAAAACCCATTTTCGCTATAAATAAAA
GGTAACTGTACAAAATAAGTACAAT

or a portion thereof or a degenerate variant thereof.

4. A recombinant DNA molecule as defined in any one of claims 1 to 3 which further comprises regulatory sequences operably linked to the structural gene for the IL-2 β chain or portion thereof.

5. A recombinant DNA molecule as defined in claim 4 which is a plasmid.

6. A recombinant DNA molecule as defined in claim 5, this being one of the following

pIL-2R β 6,

pIL-2R β 9,

pIL-2R β 19,

pIL-2R β 30.

7. A host cell which has been transformed by a recombinant DNA molecule as defined in any one of claims 1 to 6.

8. A host cell as defined in claim 7, which is a bacterial cell or a yeast cell or a mammalian cell.

9. A protein having the structure defined by the structural gene set forth in claim 2 or a portion thereof.

10. A hybridoma, sub-clone or mutant thereof capable of secreting a monoclonal antibody having a specific affinity to a protein as defined in claim 9.

11. A monoclonal antibody having a specific affinity to a protein as defined in claim 9.

12. A method of producing a hybridoma as defined in claim 10 which comprises immunizing a non-human animal with a protein as defined in claim 9, removing spleen cells from the immunized animal and fusing the spleen cells with non-immunoglobulin secreting myeloma cells, and selecting from the resulting hybridomas a cell line which produces a monoclonal antibody having the desired binding specificity and, if desired, subsequently sub-cloning said hybridoma.

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Not eligible for priority
Nouvellement déposée

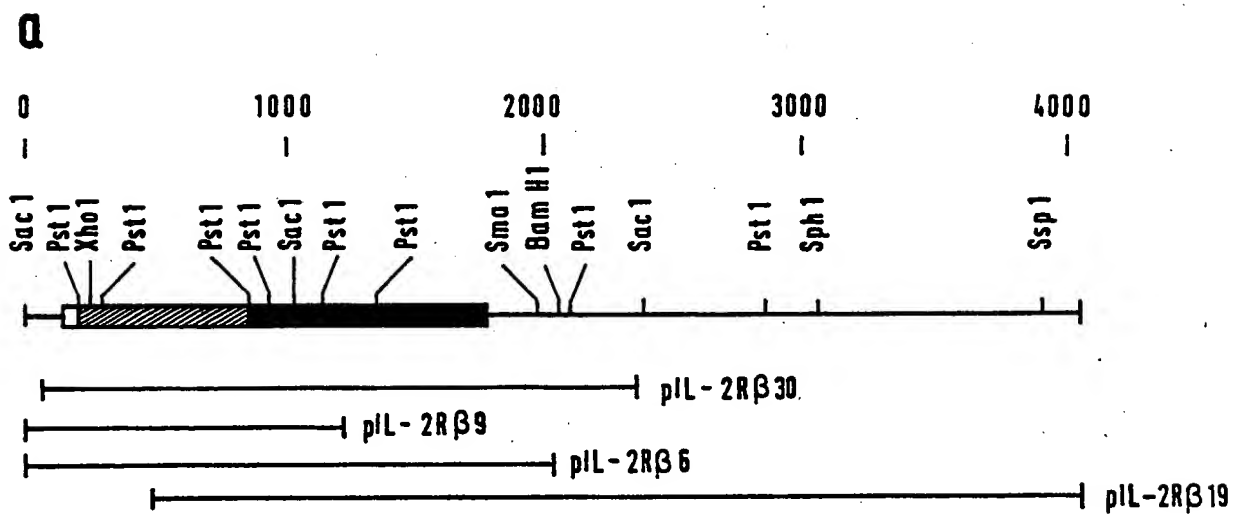


FIG. 1a

Fig. 1b

GCAGCCAGAGCTCAGCAGGGCCCTGGAGAGATGGCCA														37
CGGTCCCAGCACCGGGGAGGACTGGAGAGCGCGCGCTGCCACCGCCCCA														
	TGTCTCAGCCAGGGCTTCCTTCCTCGGCTCCACCCTGTGGATGTA <u>ATG</u>													134
														Met
-25	GCG	GCC	CCT	GCT	CTG	TCC	TGG	CGT	CTG	CCC	CTC	CTC	ATC	173
	Ala	Ala	Pro	Ala	Leu	Ser	Trp	Arg	Leu	Pro	Leu	Leu	Ile	
-12	CTC	CTC	CTG	CCC	CTG	GCT	ACC	TCT	TGG	GCA	TCT	GCA	GCG	212
	Leu	Leu	Leu	Pro	Leu	Ala	Thr	Ser	Trp	Ala	Ser	Ala	Ala	
2	GTG	AAT	GCC	ACT	TCC	CAG	TTC	ACA	TGC	TTC	TAC	AAC	TCG	251
	Val	<u>AsN</u>	<u>Gly</u>	<u>Thr</u>	Ser	GIN	Phe	Thr	<u>Cys</u>	Phe	Tyr	AsN	Ser	
15	AGA	GCC	AAC	ATC	TCC	TGT	CTC	TGG	AGC	CAA	GAT	GGG	GCT	290
	Arg	Ala	<u>AsN</u>	<u>Ile</u>	<u>Ser</u>	<u>Cys</u>	Val	Trp	Ser	GIN	Asp	Gly	Ala	
28	CTG	CAG	GAC	ACT	TCC	TGC	CAA	GTC	CAT	GCC	TGG	CCG	GAC	329
	Leu	GIN	Asp	Thr	Ser	<u>Cys</u>	GIN	Val	His	Ala	Trp	Pro	Asp	
41	AGA	CGG	CGG	TGG	AAC	CAA	ACC	TGT	GAG	CTG	CTC	CCC	GTG	368
	Arg	Arg	Arg	Trp	<u>AsN</u>	<u>GIN</u>	<u>Thr</u>	<u>Cys</u>	Glu	Leu	Leu	Pro	Val	
54	AGT	CAA	GCA	TCC	TGG	GCC	TGC	AAC	CTG	ATC	CTC	GGA	GCC	407
	Ser	GIN	Ala	Ser	Trp	Ala	Cys	AsN	Leu	Ile	Leu	Gly	Ala	
67	CCA	GAT	TCT	CAG	AAA	CTG	ACC	ACA	GTT	GAC	ATC	GTC	ACC	446
	Pro	Asp	Ser	GIN	Lys	Leu	Thr	Thr	Val	Asp	Ile	Val	Thr	
80	CTG	AGG	GTG	CTG	TGC	CGT	GAG	GGG	GTG	CGA	TGG	AGG	GTG	485
	Leu	Arg	Val	Leu	<u>Cys</u>	Arg	Glu	Gly	Val	Arg	Trp	Arg	Val	
93	ATG	GCC	ATC	CAG	GAC	TTC	AAG	CCC	TTT	GAG	AAC	CTT	CGC	524
	Met	Ala	Ile	GIN	Asp	Phe	Lys	Pro	Phe	Glu	AsN	Leu	Arg	
106	CTG	ATG	GCC	CCC	ATC	TCC	CTC	CAA	GTT	GTC	CAC	GTG	GAG	563
	Leu	Met	Ala	Pro	Ile	Ser	Leu	GIN	Val	Val	His	Val	Glu	
119	ACC	CAC	AGA	TGG	AAC	ATA	AGC	TGG	GAA	ATC	TCC	CAA	GCC	602
	Thr	His	Arg	<u>Cys</u>	<u>AsN</u>	<u>Ile</u>	<u>Ser</u>	Trp	Glu	Ile	Ser	GIN	Ala	
132	TCC	CAC	TAC	TTT	GAA	AGA	CAC	CTG	GAG	TTC	GAG	GCC	CGG	641
	Ser	His	Tyr	Phe	Glu	Arg	His	Leu	Glu	Phe	Glu	Ala	Arg	
145	ACG	CTG	TCC	CCA	GGC	CAC	ACC	TGG	GAG	GAG	GCC	CCC	CTG	680
	Thr	Leu	Ser	Pro	Gly	His	Thr	Trp	Glu	Glu	Ala	Pro	Leu	
158	CTG	ACT	CTC	AAG	CAG	AAG	CAG	GAA	TGG	ATC	TGC	CTG	GAG	719
	Leu	Thr	Leu	Lys	GIN	Lys	GIN	Glu	Trp	Ile	<u>Cys</u>	Leu	Glu	
171	ACG	CTC	ACC	CCA	GAC	ACC	CAG	TAT	GAG	TTT	CAG	GTG	CGG	758
	Thr	Leu	Thr	Pro	Asp	Thr	GIN	Tyr	Glu	Phe	GIN	Val	Arg	
184	GTC	AAG	CCT	CTG	CAA	GGC	GAG	TTC	ACG	ACC	TGG	AGC	CCC	797
	Val	Lys	Pro	Leu	GIN	Gly	Glu	Phe	Thr	Thr	Trp	Ser	Pro	

Fig. 1b cont'd

197	TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC	836
	Trp Ser Gln Pro Leu Ala Phe Arg Thr Lys Pro Ala Ala	
210	CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC	875
	Leu Gly Lys Asp Thr <u>Ile Pro Trp Leu Gly His Leu Leu</u>	
223	GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG	914
	<u>Val Gly Leu Ser Gly Ala Phe Gly Phe Ile Ile Leu Val</u>	
236	TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG	953
	<u>Tyr Leu Leu Ile</u> AsN <u>Cys</u> Arg AsN Thr Gly Pro Trp Leu	
249	AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG	992
	Lys Lys Val Leu Lys <u>Cys</u> AsN Thr Pro Asp Pro Ser Lys	
262	TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC	1031
	Phe Phe Ser Gln Leu Ser Ser Glu His Gly Gly Asp Val	
275	CAG AAG TGG CTC TCT TCG CCC TTC CCC TCA TCG TCC TTC	1070
	Gln Lys Trp Leu Ser Ser Pro Phe Pro Ser Ser Ser Phe	
286	AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA	1109
	Ser Pro Gly Gly Leu Ala Pro Glu Ile Ser Pro Leu Glu	
301	GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG	1148
	Val Leu Glu Arg Asp Lys Val Thr Gln Leu Leu Leu Gln	
314	CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC	1187
	Gln Asp Lys Val Pro Glu Pro Ala Ser Leu Ser Ser <u>AsN</u>	
327	CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC	1226
	<u>His Ser Leu</u> Thr Ser <u>Cys</u> Phe Thr AsN Gln Gly Tyr Phe	
340	TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC	1265
	Phe Phe His Leu Pro Asp Ala Leu Glu Ile Glu Ala <u>Cys</u>	
353	CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC	1304
	Gln Val Tyr Phe Thr Tyr Asp Pro Tyr Ser Glu Glu Asp	
366	CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC	1343
	Pro Asp Glu Gly Val Ala Gly Ala Pro Thr Gly Ser Ser	
379	CCC CAA CCC CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC	1382
	Pro Gln Pro Leu Gln Pro Leu Ser Gly Glu Asp Asp Ala	
392	TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC	1421
	Tyr <u>Cys</u> Thr Phe Pro Ser Arg Asp Asp Leu Leu Leu Phe	
405	TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT	1460
	Ser Pro Ser Leu Leu Gly Gly Pro Ser Pro Pro Ser Thr	

Fig. 1b cont'd

418	GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC	1499
	Ala Pro Gly Gly Ser Gly Ala Gly Glu Glu Arg Met Pro	
431	CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC	1538
	Pro Ser Leu Gln Glu Arg Val Pro Arg Asp Trp Asp Pro	
444	CAG CCC CTG GGG CCT CCC ACC CCA GGA GTC CCA GAC CTG	1577
	Gln Pro Leu Gly Pro Pro Thr Pro Gly Val Pro Asp Leu	
457	GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG	1616
	Val Asp Phe Gln Pro Pro Pro Glu Leu Val Leu Arg Glu	
470	GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA	1655
	Ala Gly Glu Glu Val Pro Asp Ala Gly Pro Arg Glu Gly	
483	GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG	1694
	Val Ser Phe Pro Trp Ser Arg Pro Pro Gly Gln Gly Glu	
496	TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT	1733
	Phe Arg Ala Leu Asn Ala Arg Leu Pro Leu Asn Thr Asp	
509	GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA	1772
	Ala Tyr Leu Ser Leu Gln Glu Leu Gln Gly Gln Asp Pro	
522	ACT CAC TTG GTG TAG ACAGATGGCCAGGGTGGGAGGCAGGCAGCT	1817
	Thr His Leu Val ***	
	GCCTGCTCTGCGCCGAGCCTCAGAAGGACCCTGTTGAGGGTCCTCAGTCCA	1868
	CTGCTGAGGACAC TCAGTGTCCAGTTGCAGCTGGACTTCTCCACCCGGAT	1918
	GGCCCCCACCAGTCCTGCACACTTGGTCCATCCATTTCCAAACCTCCACT	1969
	GCTGCTCCCGGGTCCTGCTGCCCCGAGCCAGGAAGTGTGTGTGTTGCAGGGG	2020
	GGCAGTAAGTCCCCAACTCCCTCGTTAATCACAGGATCCCACGAATTTAGG	2071
	CTCAGAAGCATCGCTCCTCTCCAGCCCTGCAGCTATTCACCAATATCAGTC	2122
	CTCGCGGCTCTCCAGGGCTCCCTGCCCTGACCTCTTCCCTGGGTTTCTGC	2173
	CCCAGCCTCCTCCTTCCCTCCCCTCCCCGTCCACAGGGCAGCCTGAGCGTG	2224
	CTTTCCAAAACCCAAATATGGCCACGCTCCCCCTCGGTTCAAAACCTTGCA	2275
	CAGGTCCCACTGCCCTCAGCCCCACTTCTCAGCCTGGTACTTGTACCTCCG	2326
	GTGTGCGTGTGGGGACATCCCCCTTCTGCAATCCTCCCTACCGTCCTCCCGAG	2377
	CCACTCAGAGCTCCCTCACACCCCCTCTGTTGCACATGCTATTCCCTGGGG	2428

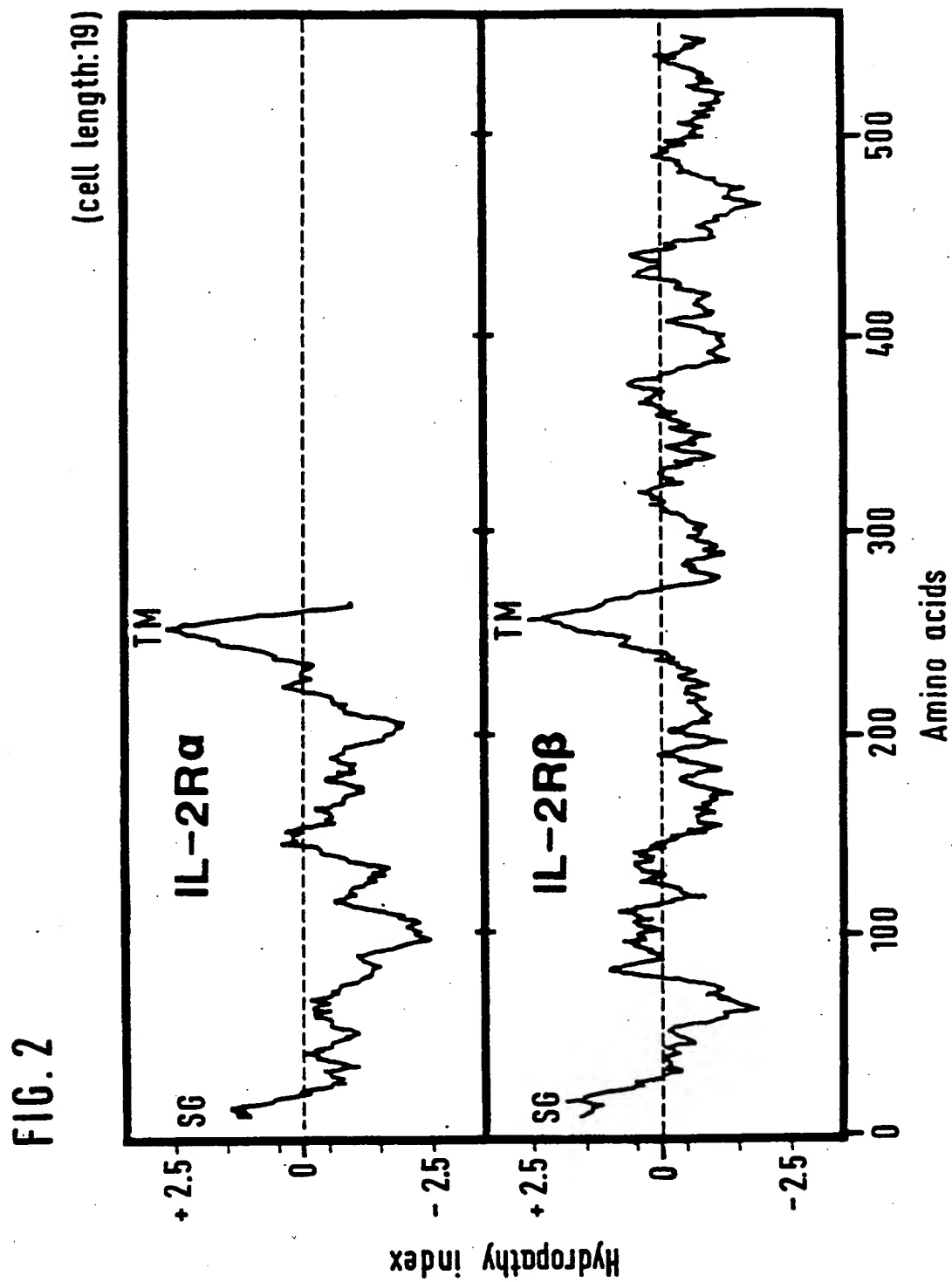
Fig. 1b cont'd

CTGCTGTGCGCTCCCCCTCATCTAGGTGACAACTTCCCTGACTCTTCAAG 2479
 TGCCGGTTTTGCTTCTCCTGGAGGGAAGCACTGCCTCCCTTAATCTGCCAG 2530
 AAACCTTCTAGCGTCAAGTGTGGAGGGAGAAGCTGTCAGGGACCCAGGGCGC 2581
 CTGGAGAAAGAGGCCCTGTTACTATTCTTTGGGATCTCTGAGGCCTCAGA 2632
 GTGCTTGGCTGCTGTATCTTTAATGCTGGGGCCCAAGTAAGGGCACAGATC 2683
 CCCCCACAAAGTGGATGCCTGCTGCATCTTCCCACAGTGGCTTCACAGACC 2734
 CACAAGAGAAGCTGATGGGGAGTAAACCCTGGAGTCCGAGGCCCCAGGCAGC 2785
 AGCCCCGCCTAGTGGTGGGCCCTGATGCTGCCAGGCCTGGGACCTCCCACT 2836
 GCCCCCTCCACTGGAGGGGTCTCCTCTGCAGCTCAGGGACTGGCACACTGG 2887
 CCTCCAGAAGGGCAGCTCCACAGGGCAGGGCCTCATTATTTTCACTGCCC 2938
 CAGACACAGTGCCCAACACCCCGTCGTATACCCTGGATGAACGAATTAATT 2989
 ACCTGGCACCACTCGTCTGGGCTCCCTGCGCCTGACATTCACACAGAGAG 3040
 GCAGAGTCCCGTGCCCATTAGGTCTGGCATGCCCCCTCCTGCAAGGGGCTCA 3092
 ACCCCCTACCCCGACCCCTCCACGTATCTTTCCTAGGCAGATCACGTTGCAA 3144
 TGGCTCAAACAACATTCCACCCCA GCAGGACAGTGACCCCAAGTCCCAGCTAA 3196
 CTCTGACCTGGGAGCCCTCAGGCACCTGCACTTACAGGCCTTGCTCACAGCT 3248
 GATTGGGCACCTGACCACACGCCCCCACAGGCTCTGACCAGCAGCCTATGAG 3300
 GGGGTTTGGCACCAAGCTCTGTCCAATCAGGTAGGCTGGGCCTGAACTAGCC 3352
 AATCAGATCAACTCTGTCTTGGGCGTTTGAACCTCAGGGAGGGAGGCCCTTGG 3404
 GAGCAGGTGCTTGTGGACAAGGCTCCACAAGCGTTGAGCCTTGGAAGGTAG 3456
 ACAAGCGTTGAGCCACTAAGCAGAGGACCTTGGGTTCCCAATACAAAAATAC 3508
 CTACTGCTGAGAGGGCTGCTGACCATTGGTCAGGATTCCTGTTGCCTTTAT 3560
 ATCOAAAATAAACTCCCCTTCTTGAGGTTGTCTGAGTCTTGGGTCTATGCC 3612
 TTGAAAAAAGCTGAATTATTGGACAGTCTCACCTCCTGCCATAGGGTCCTGA 3664
 ATGTTTCAGACCACAAGGGGCTCCACACCTTTGCTGTGTGTTCTGGGGCAAC 3716

Fig. 1b cont'd

CTACTAATCCTCTCTGCAAGTCGGTCTCCTTATCCCCCAAATGGAAATT 3766
GTATTTGCCTTCTCCACTTTGGGAGGCTCCCACTTCTTGGGAGGGTTACA 3816
TTTTTTAAGTCTTAATCATTGTGACATATGTATCTATACATCCGTATCTT 3867
TTAATGATCCGTGTGTACCATCTTTGTGATTATTTCTTAATATTTTTTCT 3918
TTAAGTCAGTTCATTTTCGTTGAAATACATTTATAAAGAAAAATCTTTGTT 3969
ACTCTGTAAATGAAAAAACCCATTTTCGCTATAAATAAAGGTAAGTGTAC 4020
AAAATAAGTACAAT 4034

Neu eingereicht / Newly filed
Nouvellement déposé



Neu eingereicht / Newly filed
Nouvellement déposé

FIG. 3a

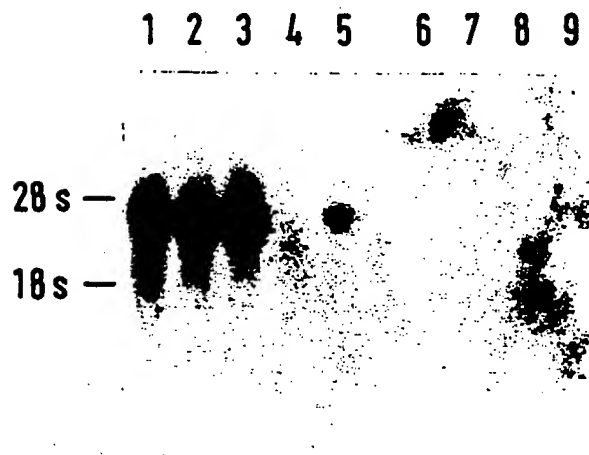
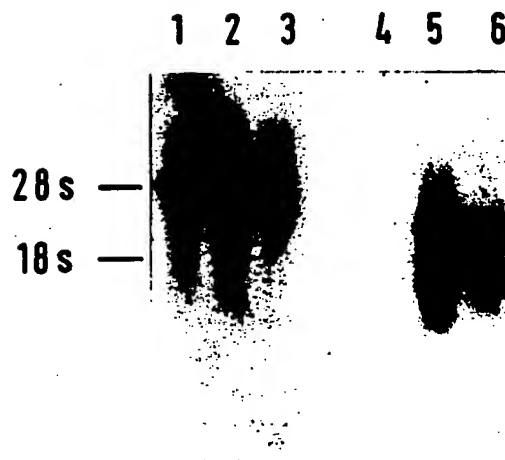


FIG. 3b



Nouvellement déposé / Newly filed
Nouvellement déposé

FIG. 4a

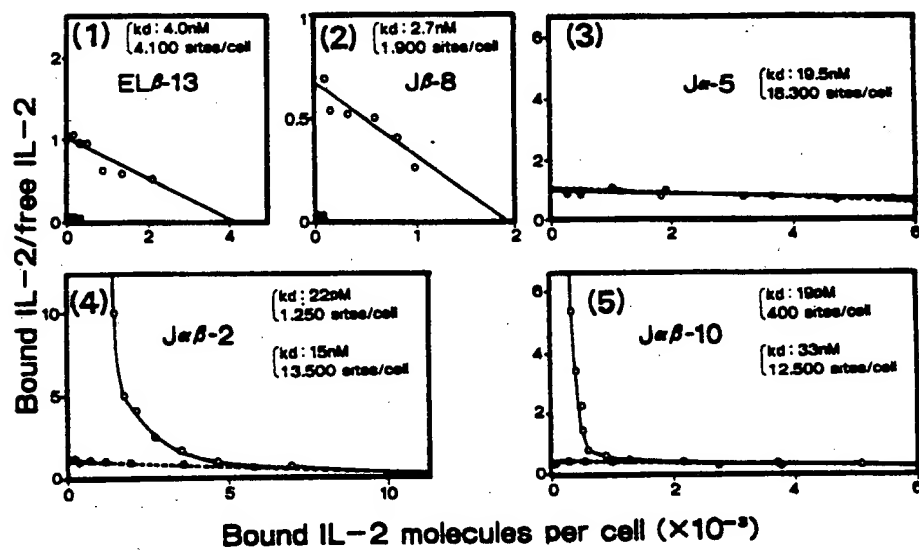
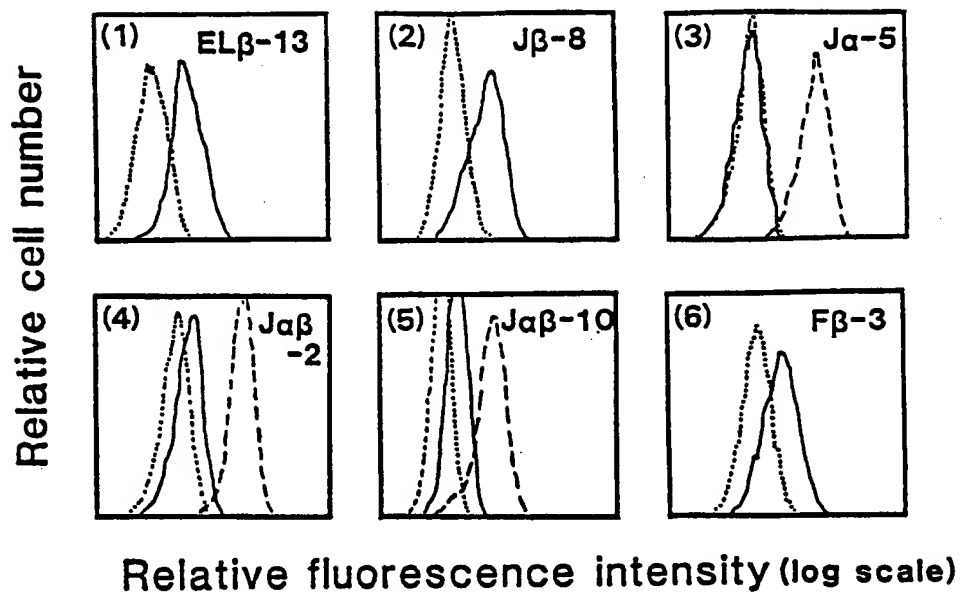


FIG. 4b

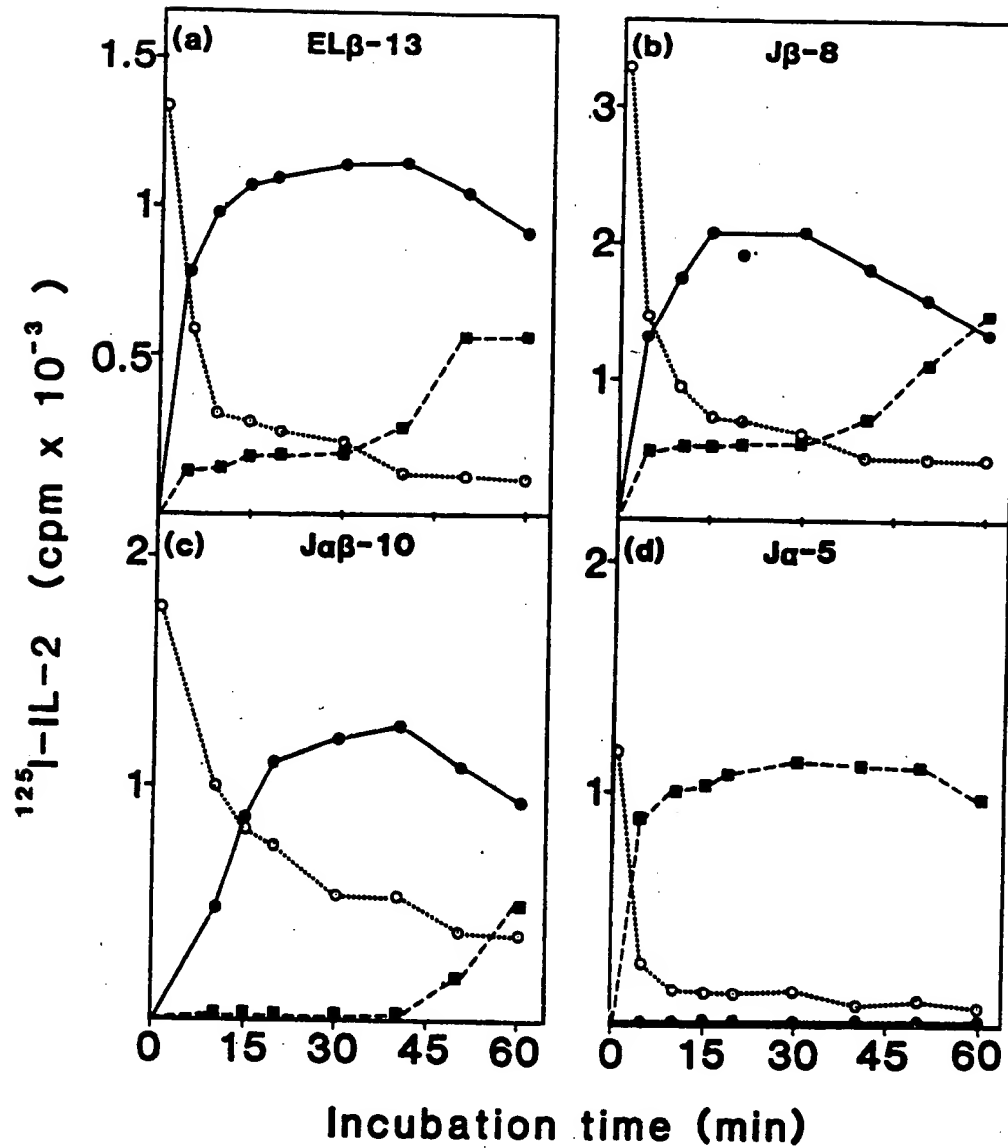
Nouvellement déposé

FIG. 5



Nouvellement déposé / Newly filed

FIG. 6





DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
X	WO-A-8 900 168 (THE UNITED STATES OF AMERICA) * Page 6, line 1 - page 8, line 30 *	9,10	C 12 N 15/00 C 12 P 21/02
Y	---	1-8	C 12 N 5/00 C 12 P 21/00 //
Y	EP-A-0 162 699 (IMMUNEX CORP.) ---	1-8	(C 12 P 21/00 C 12 R 1:91)
T	SCIENCE, vol. 244, 5th May 1989, pages 551-556, Washington, DC, US; M. HATAKEYAMA et al.: "Interleukin-2 receptor beta chain gene: generation of three receptor forms by cloned human alpha and beta chain cDNA's" * Whole document *	1-10	
T,D	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, USA, vol. 86, March 1989, pages 1982-1986, Washington, DC, US; M. TSUDO et al.: "Characterization of the interleukin 2 receptor beta chain using three distinct monoclonal antibodies" * Page 1982: "Materials and methods" *	9,10	
A	JOURNAL OF EXPERIMENTAL MEDICINE, vol. 165, January 1987, pages 223-238, The Rockefeller University Press, New York, US; K. TESHIGAWARA et al.: "Interleukin 2 high-affinity receptor expression requires two distinct binding proteins" * Page 223, lines 6-9 *		C 12 N C 12 P
			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
The present search report has been drawn up for all claims.			
Place of search THE HAGUE		Date of completion of the search 21-08-1989	Examiner VAN PUTTEN A.J.
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			



☒ CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,
namely claims:
- ☒ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

☐ LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid,
namely claims:
- ☐ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims,
namely claims: